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EMBRYOS UNDER CONDITIONS OF DENERVATION
OF THE UTERUS

By N. A. Samoshkina

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THE ACTION OF X-RAYS ON THE DEVELOPMENT OF RAT
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by N.A. Samoshkina

[Following is the translation of a paper by
N.A. Samoshkina* entitled "Deystviye luchey
rentgena na razvitiye zarodyshey krysy v
usloviyakh denervatsii matki" (English version
above) in Arkhiv Anatomii, Gistologii i
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of Academy of Medical Sciences, USSR, Prof. P.G. Svetlov)
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According to the data of P.G. Svetlov and G.F. Korsakova the nervous system of the maternal organism exerts a regulating influence on the development of rat embryos. The effect of disturbance of uterus denervation on embryogenesis studied without combination of other agents (1954 and 1957) or in combination with the action of increased temperature on the pregnant animal (1954). In the plan of this work it was of interest to detect how the uterus denervation is reflected on the changes in the development of embryos observed after the action of X-rays on pregnant animals.

The combination of denervation with the action of X-rays was of special interest also because in the literature after this time most researchers (Wilson and Karr 1951, L.B. Russell and W.L. Russell 1954, E.Ya. Grayevskiy and M.I. Shapiro, A.A. Neyfakh 1957, and others) considered that in the action mechanism of ionizing radiation on embryos with total-body irradiation of the pregnant animals, only the direct action of radiation on the embryo is of consequence. However, in recent years some authors (N.A. Kalinina 1956, E.F. Isachenko 1956,

and P.G. Svetlov and G.V. Korsakova 1957), without denying the fact of the great sensitivity of embryos, consider that the maternal organism also has an effect in the development of pathological changes observed in embryos when pregnant females were irradiated. In relation with this as the result of experiments on the effect of X-rays on embryogenesis in the conditions of uterus denervation, data should have been obtained which would confirm this or the other point of view.

Materials and methods

The research was undertaken with rats. A method developed by Prof. P.G. Svetlov and G.V. Korsakova (1954 and 1957) was applied for the operation of disrupting vegetative uterus innervation. Operations were made under ether narcosis, and through the middle cut of the abdominal cavity the following nerve sets were removed in series:

(1) Small vascular nerve (n. splanchnicus minor) with lower mesometrium ganglion (g. Mesentericus inferior) and hypogastric nerves outgoing from it (n. hypogastricus) with accessory ganglions (g. accessorius); (2) Sympathetic trunk (tr. sympathicus) in the lumbar region with the

extent 1-1.5 cm with 1-2 ganglions; (3) Celiac plexus (pl. coeliacus). Removal of the above mentioned parts of the autonomic nervous system, naturally could not completely denervate the uterus, as we did not interfere at all the nerve trunks in going to it through the sacral plexus, and innervation from the direction of sympathetic trunk was not made completely discontinuous by us. As a result of this surgery, a partial uterus denervation set in, since the largest part of afferent and also efferent nerve sets was disrupted which connect the peripheral parts of neural elements in the uterus with their corresponding central sections.

Experiments were made with uterus denervation long before pregnancy (from one to two months), and with denervation on the first day of pregnancy.

Animal irradiation was made with dosages of 60 and 200 r on the fourth day of pregnancy. This day was selected by us, resulting from the established data of our laboratory, that first, the fourth day is the peak of the sensitivity curves for rat embryos, and that second, the regulating influences on embryogenesis from the nervous

system of the maternal organism are the strongest at this time. It should be noted that at irradiation time the rat embryos were in the oviducts in the middle stages of ovum cleavage (8-16 blastomeres). A single total-body irradiation of the animals was made with RUN-3 equipment, with current voltage 180 kv, current 15 ma, rate 30r/minute, focal distance 40 cm, filters 0.5 mm Cu and 1 mm Al.

The level of radiation sickness development in experimental animals was judged from the pattern of the blood, analysis of which was made three times. Blood was taken before irradiation, on the third day and then on the seventh day after irradiation. The number of leukocytes was counted. The obtained results show that by the third day after irradiation an overt leukopenia was developed in the animals, which gradually leveled off by the seventh day, and sometimes later. The number of leukocytes in the blood of experimental animals were on the average: before irradiation - $16,900/\text{mm}^3$, 3 days after irradiation - $7,500/\text{mm}^3$, and 7 days after irradiation - $12,100/\text{mm}^3$.

The following series of experiments were made:

(1) Denervation before pregnancy with the influence of X-rays at 60 r on the fourth day of pregnancy.

(2) The same with X-ray influence at 200 r.

(3) Uterus denervation on the first day of pregnancy and irradiation of 200 r on the fourth day.

At the same time control experiments were made: (a) with X-ray influence of 60 r on the fourth day of pregnancy without denervation; (b) the same with 200 r; (c) uterus denervation on first day of pregnancy without irradiation; (d) control laparotomy without denervation on the first day in combination with X-rays on the 4th day. Embryos were investigated on the 10th day of pregnancy. The abdominal cavity was cut open and inspection of the uterus and the embryos inside it were made with live rats. In addition to this attention was paid to the external form of the placentas, their vascularization, mutual arrangement, and also their arrangement with respect to the uterine axis. After this the whole uterus was cut out and fixed in Bouin's or Vasyutichkin-Brank fluid. With the aid of a binocular dissecting microscope

NVS-1 examination of placenta were made on thick cross-sections. For this, placentas were cut by a razor approximately in the middle and the obtained slices were looked through under a microscope in reflected light.

With each dissection the following were considered: (1) percentage of implanted blastocysts, by counting the number of yellow bodies in the ovaries and the number of formed placentas; (2) number of normally-developing embryos; (3) number of anomalous embryos and character of their anomalies; (4) number of dead embryos.

Cases deserving special attention, were subjected to histological study, for which the material was mounted in paraffin and wax-embedded by usual histological methods (hematoxylin-eosin, azure II-eosin, Haedenhein's iron hematoxylin, and others). The material taken from control animals was also studied with the same methods.

The given illustrations were made by photographing the samples with apparatus "Progress" with lens magnification (microfara 1.5 and 4.5 cm) and with microscope ob. 10 and ok. 5.

Experimental results

If the disruption of the uterine innervation sets were made on non-pregnant females, and the pregnancy came after one to three months after operation, then after irradiation of maternal organism on the 4th day of pregnancy the development of embryo does not change more than with irradiation without uterine denervation. In experiments with denervation and irradiation with 60 r the received data does not differ essentially from the control data, i.e., from the results of irradiation with 60 r without uterine denervation, and these and the other data according to the percentage of implanted embryos, the number of normal and anomalous embryos were not different from those in normal animals, or these differences were statistically unreliable. We will note, that P.G. Svetlov and G.F. Korsakova (1957) in 17 day old fetuses observed deviations from the norm of female irradiation on the 4th day of pregnancy with dosages beginning from 30 r. Apparently, after irradiation with a dose of 60 r, the mentioned anomalies are not able to appear by the 10th day of pregnancy.

By increasing the radiation dosage up to 200 r with the same experimental conditions, the number of normally-developing embryos decreases to 40.6%, whereupon the number of dead embryos increased markedly.

In the series of experiments made at the same time with animal irradiation with 200 r without denervation, normal embryos constituted only 40% of the total number; the obtained numbers on dead and anomalous embryos which also differed greatly (Table 1). Analogous data was obtained by P.G. Svetlov and G.F. Korsakova (1957) in experiments with rat irradiation on the 4th day of pregnancy with 200 r. Some differences in the stated numbers are explained by the fact that these authors considered their experimental results at a later stage of uterine development.

This gives us the right to conclude that the uterine denervation made by us long before the onset of pregnancy has not any noticeable effects on the development course of embryos. This result can be explained by the fact that by the moment of onset of pregnancy, the regulation of disturbances was made, brought by the

Experiment	No. fo- male	No. yellow- plumaged died on	No. in- cubated	No. of inf.	Fetus					
					Normal		Abnormal		Dead	
					Abs.	%	Abs.	%	Abs.	%
Uterine denervation before pregnancy	18	148	100	89.9	54	40.6	15	11.3	64	45.1
+200 r....	19	107	100	93.5	45	40.0	7	7.0	55	53.0
-200 r....										
Difference and difference error*				3.6± 2.5		0.6± 0.6		4.3± 2.7		4.9± 0.6

Table 1. Uterine denervation irradiation on 4th day of pregnancy.

* The difference error is calculated by the formula

$mdiff = \sqrt{m_1^2 + m_2^2}$, where m_1 and m_2 - errors of average values in the experimental and control data; they are determined from the formula $m = \sqrt{\frac{p(100-p)}{n}}$, where p - the studied quantity in % and n - number of observations.

innervation set. Therefore, we have tried to bring closer the times of uterine denervation and the beginning of pregnancy.

With this purpose we made experiments with rat irradiation on the 4th day of pregnancy after uterus denervation, which was made on the 1st day of pregnancy (immediately after the discovery of sperm cells in the vaginal smear of the females, which were placed the day before in the same cage with males).

The data of these experiments have somewhat different character and deserve a more detailed explanation. In order to know what influence has one uterine denervation on the development, experiments were made, where the operation for the disruption of vegetative uterine innervation was made on the 1st day of pregnancy. On the 10th day the rats were sacrificed, and their embryos were carefully studied. It turned out that with one denervation, made on the 1st day of pregnancy without subsequent irradiation, essential deviations in the development from the norm were immediately cleared up. As seen from Table 2, the number of ineffective implantations

with respect to the norm increases.

Experiment	Fetus									
	Norm		Anom.		Dead		Total		%	
	Abs.	%	Abs.	%	Abs.	%	Abs.	%	Abs.	%
Denervation on 1st day...	10	100	88	76.2	55	57.0	31	55.8	15	17.2
Norm.....	9	91	76	81.0	67	72.3	3	3.0	0	7.9
Difference and difference error				15.4 ± 3.1		31.2 ± 6.2		21.0 ± 5.04		9.3 ± 4.9

Table. 2. Uterine denervation on the first day of pregnancy and normal development.

The Implantation percentage decreased to 76.2%, while in normal rats, according to the data of our laboratory, 91.6% of the ova are implanted. The number of normally-developing embryos decreases to 57% (difference with respect to control is 31.2 ± 6.2). The number of developmental anomalies increases to 55.8%. The obtained data fully agrees with the results of analogous experiments by P.G. Svetlov and G.F. Korsakova (1957), in which the uterine denervation on the first day of pregnancy also considerably increased the number of ineffective

implantations. Considerable effect was exerted by the operation on the development of the prior implanted blastocyst: by the 10th day of pregnancy only 75.6% of the implanted embryos turned out to be normal. In our experiments the number of normally-developing embryos after the operation was lower. As seen from Table 2, it reached 57.0%. These characteristics evidently are explained by the individual differences of experimental animals, and the conditions of their maintenance.

Thus, experiments with uterine denervation without X-raying of animals showed that the operation made on the first day involves serious disturbances in the embryogenesis of the rats.

Experiments were made with irradiation of pregnant animals on the 4th day with 200 r without uterine denervation with the purpose of clarifying the peculiarities of X-ray effects on the development. The selection of dosage was motivated by the following: 200 r is an average dosage and, judging by data from the literature its effect is considerable, but not too strong for our purposes.

The character obtained besides these deviations from the norm was somewhat different in comparison with those with denervation on the 1st day of pregnancy without irradiation. First of all X-raying did not exert any influence on the percentage of implanted blastocysts as in the norm it equaled 93.5%.

Thus, it can be considered that X-rays in the dosage applied by us do not influence the percentage of implantation in rats. This agrees with the unpublished experimental data of P.G. Svetlov and G.F. Korsakova, in which with the influence of X-rays with dosages from 30 r and up to 400 r on the pregnant animal, there were not obtained any noticeable deviations in the implantation number.

However, as shown in Table 1, from the number of implanted embryos only 40% develop normally. The remaining 60% constitute different deviations from the norm, whereupon, as seen from the Table, a large number of them die before the 10th day. Thus, the X-rays per se with this dosage cause a very serious disturbance in rat development.

Fig. 1. Quantitative relationship of the implanted embryo number with different influencing factors.

I - norm; II - uterine denervation on 1st day +200 r on the 4th day of pregnancy; III - uterine denervation on the 1st day of pregnancy; IV - irradiation of 200 r on the 4th day of pregnancy.

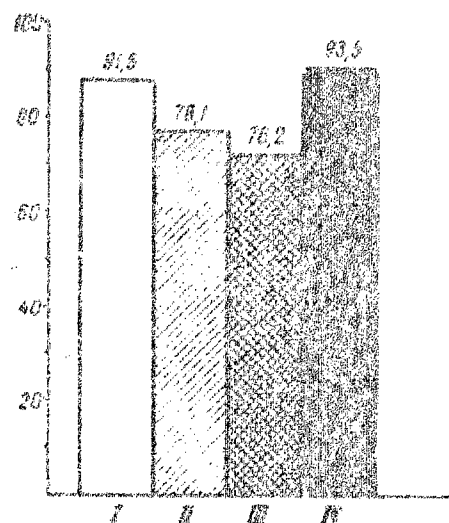
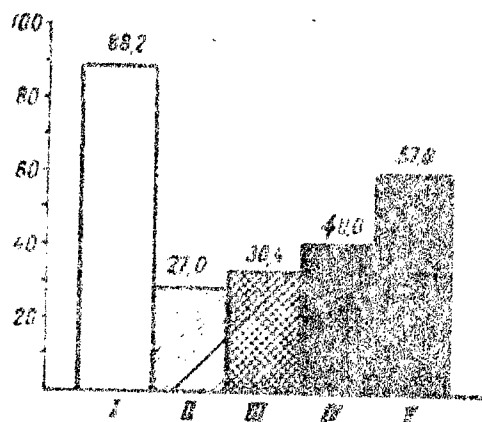


Fig. 2. Quantitative relationships (in %) of normally-developing embryos with different influencing factors on the pregnant animal.

I - norm; II - uterine denervation on the 1st day + 200 r on the 4th day of pregnancy; III - laporatomy on the 1st day + 200 r on the 4th day of pregnancy; IV - irradiation of 200 r; V - denervation on the 1st day of pregnancy.



Combination of X-ray influence with 200 r dosage on the 4th day of pregnancy and uterine denervation on 1st day of pregnancy on the rat development showed, that the percentage of implantations decreases not more than with denervation without irradiation. Percentage decrease of implantation to 78.1 occurs because of the uterine denervation made on the first day of pregnancy (Fig. 1).

The data, obtained with the consideration of normal embryo number, indicate on other things. It was clarified, that with uterine denervation on the 1st day of pregnancy and with the irradiation with 200 r on the 4th day - only 27.0% of the embryos develop normally (Table 3). Such a sharp decrease of the normal embryo number is very characteristic.

It is shown on Fig. 2, that one denervation gives 57.0% of normal embryos, X-rays with 200 r on the 4th day of pregnancy - 40.0%. With the combination of these two factors the number of normally-developing embryos is only 27.0%. On the basis of this, it can be concluded that the performed uterine denervation considerably increases the X-raying effect.

In none of the series of experiments did the number of anomalous and dead embryos increase so sharply. With denervation and irradiation they constituted 73.0% of the total embryo number.

The development of anomalies considered by us were principally represented as a lag in development, wrinkling and deformation of embryo sacs, non-closure of the primary uterine opening, incorrect arrangement of placenta with respect to the uterine opening and embryos in embryo sacs.

Experiment	No. of males	No. viable boiles	No. impi. embryos	Implant. %	Fetus					
					abs.	%	abs.	%	abs.	%
Denervation on 1st day ± on 4th 200 r.....	11	111	80	73.1	24	27.0	31	34.6	34	38.2
on 4th 200 r.....	10	107	100	93.5	40	39.0	7	7.0	53	53.0
Difference and difference error.				18.4 ± 4.2		13.0 ± 5.1		27.6 ± 5.1		19.8 ± 7.1

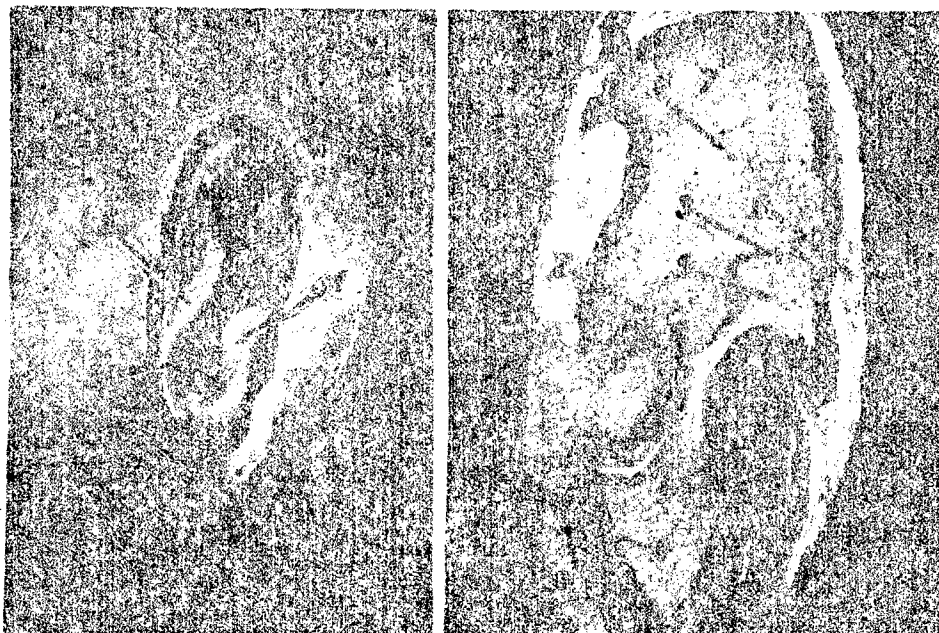
Table 3. Uterine denervation on the fifth day of pregnancy in combination with irradiation on the fourth day of pregnancy.

In our experiments, lag in development was encountered most frequently. In the norm the 10 day old embryo was in a stage of late neurula with well expressed head and allantois (Fig. 3a). On the sections the membranes were easily identifiable, the first isolate somites are seen (Fig. 3b). Among experimental embryos, embryo sacs were observed, the sizes of which were two to three times smaller than normal, of rounded form, with thick walls (Fig. 4a). The outer placenta cavity, exocoelom and amnion are well-expressed (Fig. 4b), but the embryo has been delayed much in development and is approximately in the stage of 7 1/2 to 8 days.

Incorrect locations of placentas with respect to the uterine opening were very frequently encountered among other deviations from the norm. The diagonal position of the embryo-sac with respect to decidum was frequently observed. Then the embryo itself in the embryo-sac changed its uterine position, turning around its own axis by 45 to 90° (Fig. 5).

Fig. 3. A normal 10 day old embryo. Cross-section through the placenta.

A - embryo-sac and disidium in the reflected light (Zeiss micropower 1.5 cm); B - The same greatly amplified. Fixation: Vasyutskhina-Brank fluid; staining: Hematoxylin-eosin; Microphoto. ob. 10, ok. 5. 1 - anterior and posterior of embryos; 2 - allantois; 3 - amnion wall; 4 - posterior end of embryo; 5 - amnionic cavity; 6 - exocoelom; 7 - ectoplacental cavity; 8 - yolk-sac.



Different forms of wrinkling and deformation of the embryo-sac were met rather frequently. Fig. 6A shows how this form of anomaly look on the thick cuts through the uterus with their study in the reflected light with small magnification, and fig. 6b - on histological preparation. A gradual wrinkling and death of embryos occur. It can be seen at great magnifications, that among cell elements of the embryo, cells with pyknotic nuclei are found.

In the case of embryo dead after implantation in the decidiums instead of the embryo-sac only its emascerated remainders or hemorrhages were preserved (Fig. 7a and b).

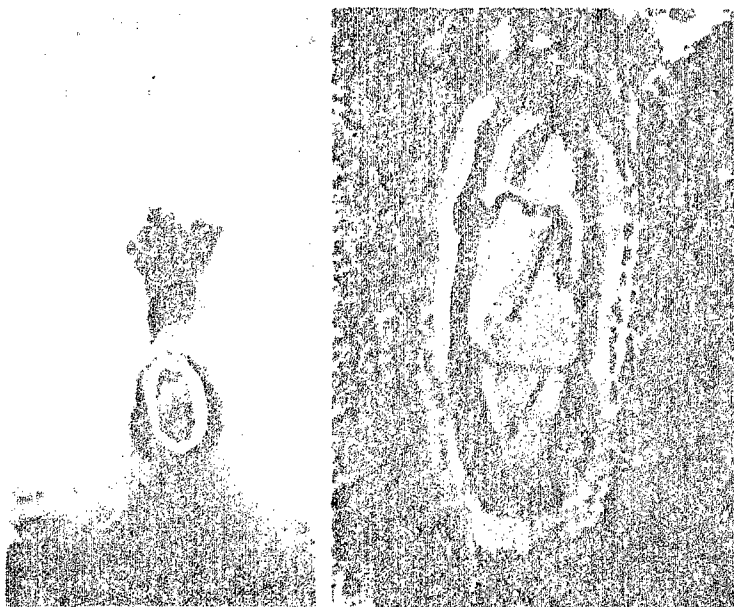
The stated anomalies were sometimes also found in control, whereupon the character of those and of the others was one and the same, but their number, as it was stated above, sharply increased in the experiment.

Thus, uterine denervation, made on the first day of pregnancy, considerably amplifies the X-raying effect, on the basis of which we can assert that the influence of maternal organism with the action of ionizing radiations on the pregnant animal is considerable.

Fig. 4. Uterine denervation on the 1st day of pregnancy with irradiation by 200 r on the 4th day.

10 day old embryo; acute lag in development. The embryo corresponds to 7 1/2-8 days of age. The embryo-sac is much better developed than the embryo. Cross-section through the placenta. A - embryo-sac in reflected light (Zeiss micropower 1.5 cm); B - the same with greater magnification. Fixation and staining - see Fig. 3b. Microphoto. od. 10, ok. 5.

1 - amnion wall; 2 - chorion; 3 - ectoplacental cone.



However, this does not say yet that the obtained amplification of X-ray effect on embryogenesis occurs just because of cutting the nerves to the uterus. To ascertain the role of narcosis, operation shock and the presence of wound in parallel with experiments on uterine denervation, experiments were conducted with control laparotomy without denervation.

In the same period, i.e., on the first day of pregnancy, laparotomy was undertaken with the animals, which was accompanied by the same manipulations, as in the operation where the disturbance of the vegetative uterus denervation. Under ether narcosis after opening up the abdominal cavity the intestine was isolated by a cloth moistened with Ringer's solution and will work at the abdominal cavity. By a careful movement of the forceps both horns of the uterus were found, in the same way after opening the peritoneum N. splanchnicus with N.N. hypogastrici leading from it were found. The disruption of their entirety was not made. Then in succession, as

in the denervation operation, approach to tr. sympathetic was freed in its lumbar region, and also to the celiac plexus. After the stated manipulations the abdominal cavity was sutured, and on the 4th day of pregnancy these animals were irradiated with 200 r in the same conditions as those for the animals in the other series. As in experiments with uterus denervation, the animals were killed on the 10th day, and the embryos were carefully studied.

It was found, that the control laparotomy somewhat increases the number of ineffective implantations with respect to the normal. However the difference in the percentage of implanted embryos those in the normal animals is statistically unreliable (difference 11.0 ± 5.3) (Table 4).

From the number of implanted embryos only 30.4% developed normally, the other 69.6% constituted the anomalous and dead group. The reality of difference with the norm in this respect cannot be doubted.

Fig. 5. Uterine denervation on the first day of pregnancy with 200 r radiation on the fourth day.

10 day old embryo-sac of a rat. Cross-section cut through placenta. Longitudinal, embryo axis displaced by 90° ; head (1) is turned to the observer, back (2) and allantois (3) - in the back. Photograph made with reflected light. (Zeiss micropower 1.5 cm).



We have not done any experiment on the influence of one laparotomy on embryogenesis. But this information is in the research of P.G. Svetlov and G.F. Korsakova (1957). After laparotomy without denervation and without animal irradiation on the 4th day of pregnancy, the percentage of implanted embryos and deviations from the norm in their further development decrease negligibly (the differences are statistically insignificant).

The comparison of obtained data on laparotomy and irradiation with the data on radiation show that control laparotomy increases the weight of X-ray effect, as with irradiation the number of normal embryos was 40%, with the same dosage, but with preliminary laparotomy on the 1st day of pregnancy, their number decreased to 30.4%.

Thus, this series of experiments showed, that operation interference itself gives a very sharp amplification of irradiation action effect on embryogenesis.

By comparing results from laparotomy and denervation on the 1st day of pregnancy it is found that their difference is negligible and statistically unreliable. The number of normally-developing embryos with denervation

and a subsequent irradiation equals 27.0%, with control laparotomy and radiation it was 30.4%. This means that the laparotomy control operation which was made by us is itself an interference which changes the maternal body so much that uterine denervation in given case almost does not add anything to it.

Experiment	No. of Males	No. of Females	No. of Total	No. of Males	Fetus				No. of Males	No. of Females	No. of Total
					abs.	%	abs.	%	abs.	%	
Laparotomy on 1st & 4th day with 200 r.m.	10	22	32	39.6	24	30.4	12	15.2	43	54.43	
None.....	3	23	26	91.6	67	86.2	3	3.9	6	7.9	
Difference and diff. error...				51.0		55.8		11.3		48.53	
				5.9		6.3		4.6		±6.4	

Table 4. Laparotomy on the first day of pregnancy (without uterine denervation).

Fig. 6. Uterine denervation on the first day of pregnancy with 200 r radiation on the fourth day.

10 day old embryo-sac of a rat. Wrinkled embryo sac. A - photograph in reflected light (Zeiss micropower 1.5 cm);

B - the same with a greater magnification. Microphoto. od. 10, ok. 5.

1 - wrinkled wall of the embryo-sac; 2 - head of the embryo; 3 - posterior of embryo.

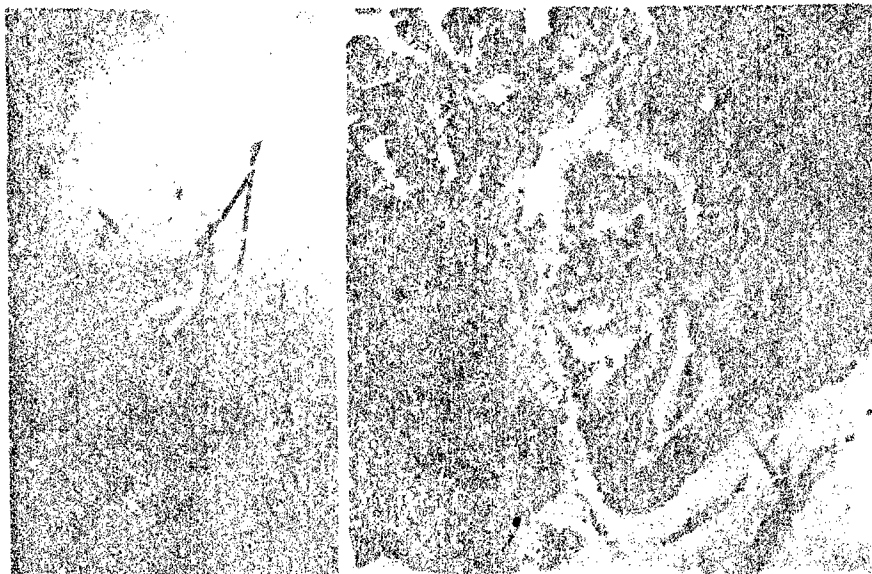
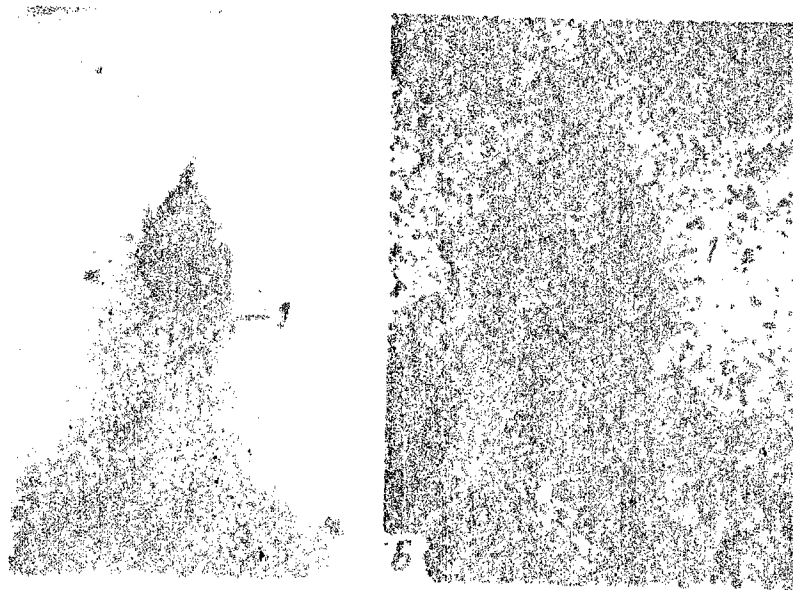


Fig. 7. Uterine denervation on the first day of pregnancy with 200 r radiation on the fourth day.

10 day old embryo-sac. Dead embryo. A - photograph with reflected light (Zeiss micropower 1.5 cm); 1 - hematoma in deciduum in place of the embryo-sac; B - the same. Microphoto. ob. 10, ok. 5.



Conclusions

1. Uterine denervation before pregnancy has no influence on irradiation effect. The obtained data on implantation, number of normal, anomalous and dead embryos in the experiment do not differ from control.

2. The uterine denervation operation, made on the 1st day of pregnancy without X-irradiation, exerts a noticeable effect on implantation and development of embryo, considerably decreasing the implantation percentage in comparison with control, and also decreasing the number of normally-developing embryos.

3. With combined effects of uterine denervation on 1st day of pregnancy and animal irradiation with 200 r on the 4th day, an amplification of the irradiation effect is observed, which is expressed in the increased number of anomalous embryos by the 10th day of development. It can be considered that the influence of the maternal nervous system is expressed by moderation of indirect X-ray action on the embryo development.

4. Laparotomy without denervation, made on the 4th day of pregnancy in combination with X-raying on the

4th day also considerably increases the X-raying effect, but less than the denervation.

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EFFECT OF X-RAYING UPON THE DEVELOPMENT OF RAT EMBRYOS UNDER CONDITIONS OF UTERUS DENERVATION

N. A. Semashina

The aim of the present investigation was to follow how the uterus denervation affects the changes in the embryo development which have been caused by the application of x-rays to the pregnant animals. Analysis of the experimental material showed that the denervation of uterus performed long before the onset of pregnancy, did not noticeably affect the course of the embryonic development. However the disturbance of the uterus innervation mechanism produced on the first day of pregnancy, influenced the development of embryos treated with x-rays on the fourth day of their development. The notable decrease in the percentage rate of implantation (up to 73.1%: under normal conditions it equals to 91.5%) and in the number of normally developing embryos (up to 37%: under normal conditions it equals to 69.2%) was revealed. On the basis of the data obtained the author comes to the conclusion that the denervation of uterus executed on the first day of pregnancy considerably enhanced the effect of x-raying and consequently allows to ascertain that the maternal organism subjected to ionizing radiation during pregnancy of the animal exerts a considerable influence upon the embryo development.

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